Figure 1.

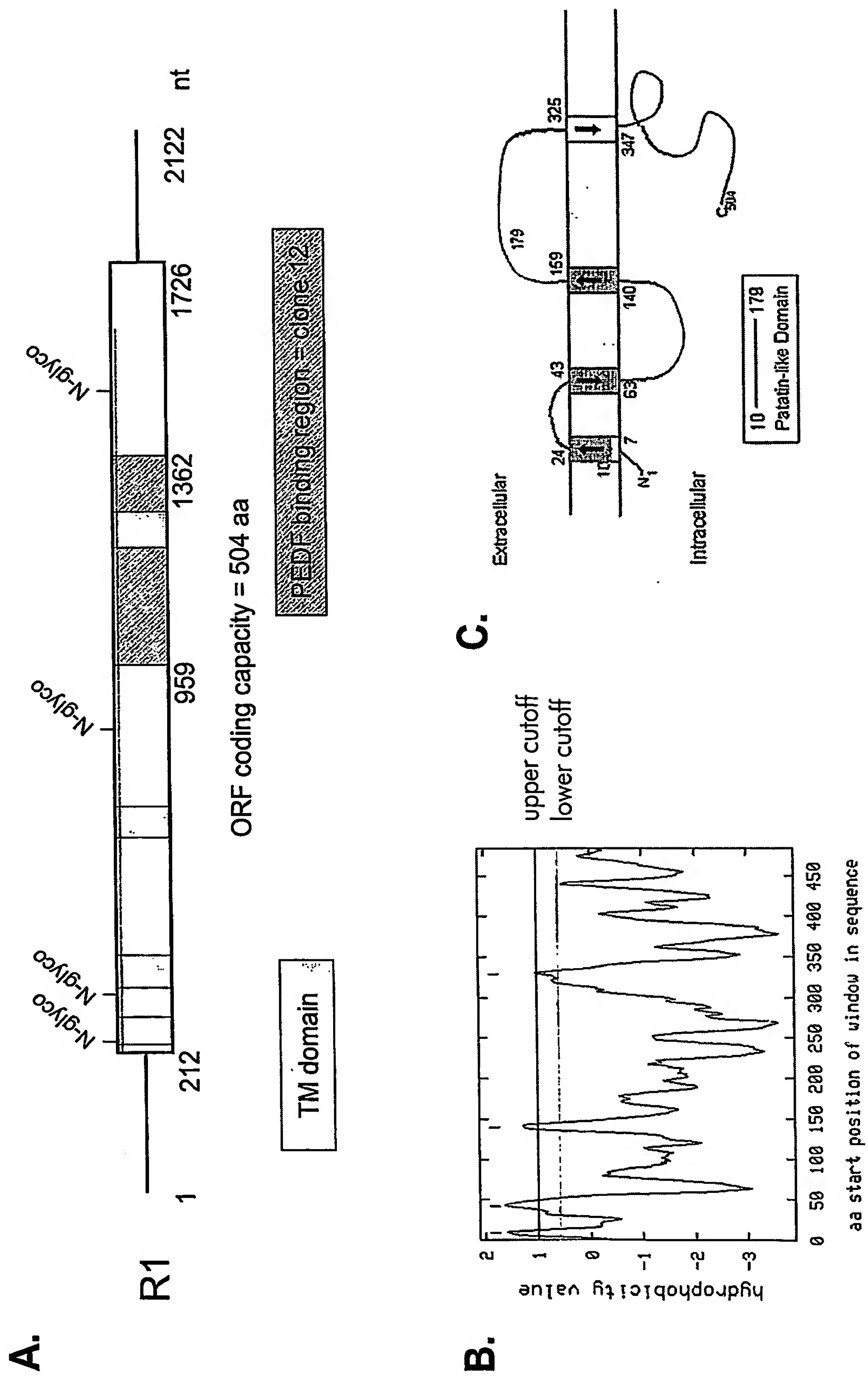
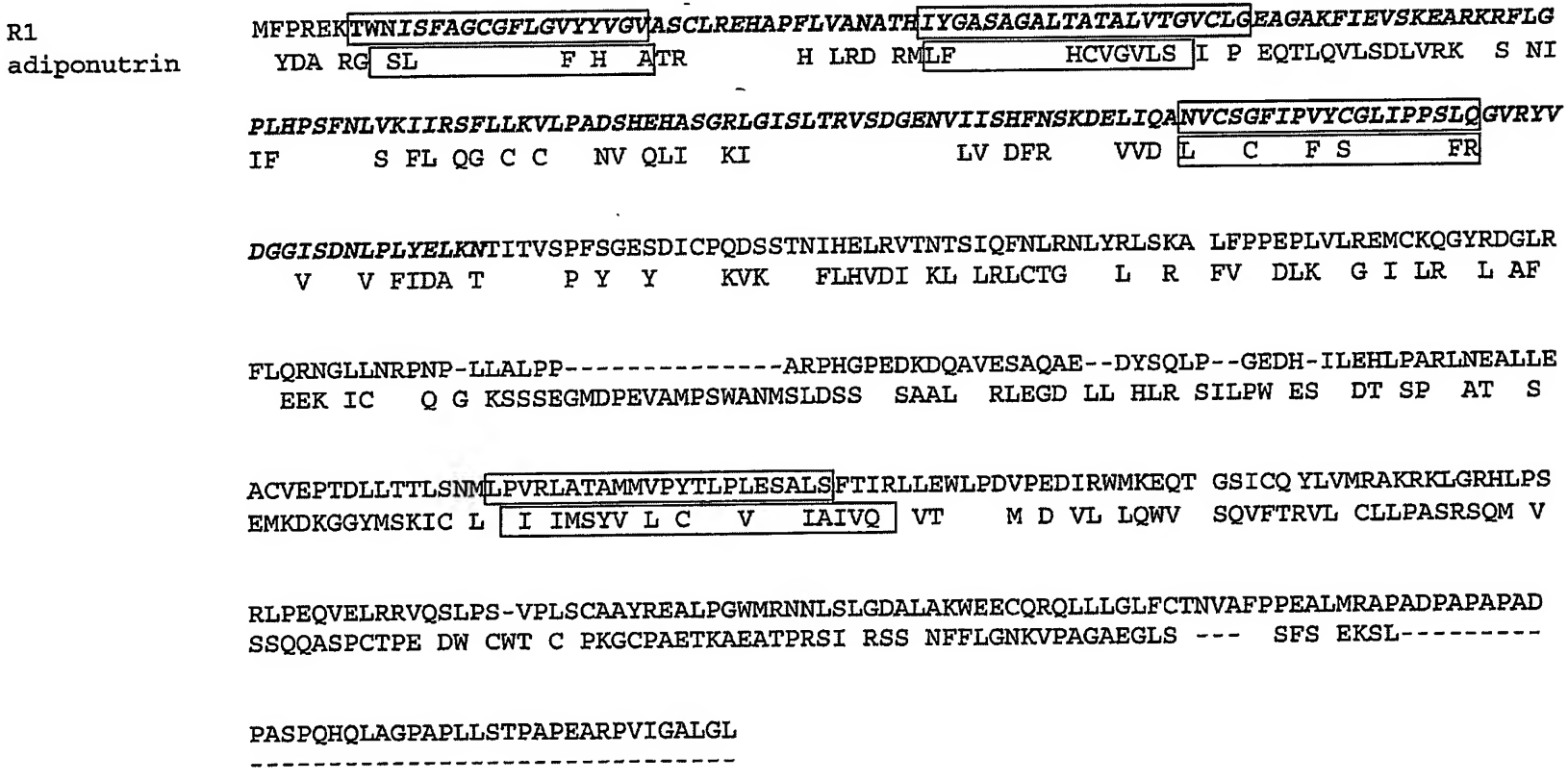

A.
R1
1
212
959
1362
1726
2122
nt
N-glyco
N-glyco
N-glyco
N-glyco
ORF coding capacity = 504 aa
TM domain
PEDF binding region = clone 12
B.
upper cutoff
lower cutoff
hydrophobicity value
aa start position of window in sequence
C.
Extracellular
Intracellular
N1
10
24
43
63
140
159
179
325
347
C504
10 —— 178
Patatin-like Domain

Figure 1.

D.



E.

253 GLLNRPN PLLALPPARP HGEPDKDQAV ESAQAEDYSQ LPGE 293

450 T NVAFPPEALR MRAPADPAPA PADPASPQHQ LAGPAPLLST PAPEARPVIG ALGL 504

Figure 1.

F.

Homologous patatin phospholipase A (PLA) active site in R1: **S**47 and **D**166

Active site serine

NA THIYGA**S**AGA LTA R1
YF DVIGGT**S**TGG LLT Patatain B2
CA TYVAGL**S**GST WYM cPLA2

Active site aspartic

SLQ GVRYV**D**GGIS DNLPLYE R1
ARY EFNLV**D**GAVA TVGDPAL Patatin B2
KSK KIHVV**D**SGL- TFNLPYP cPLA2

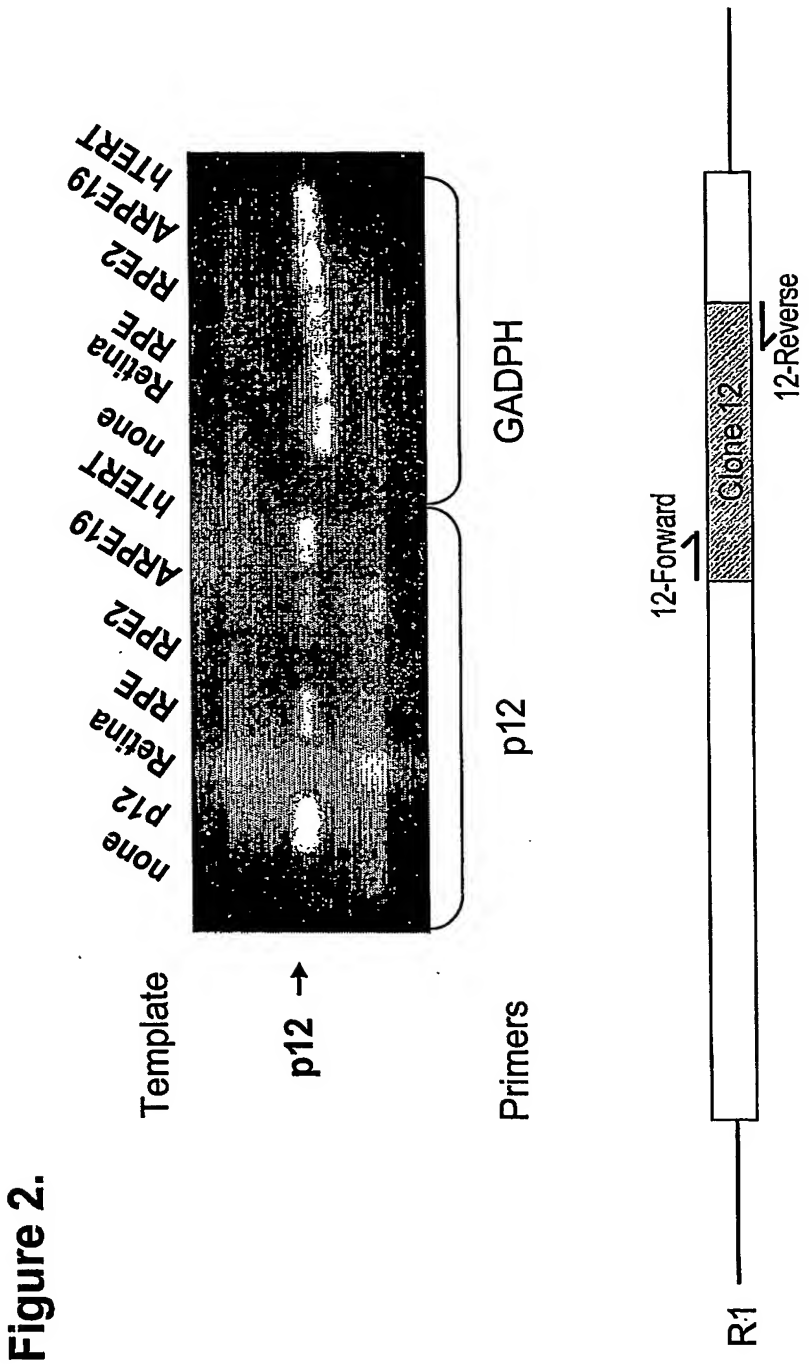

Template
p12
none
p12
Retina
RPE
RPE2
ARPE19
hTERT
none
Retina
RPE
RPE2
ARPE19
hTERT
Primers
p12
GADPH
12-Forward
Clone 12
12-Reverse
R1

Figure 2.

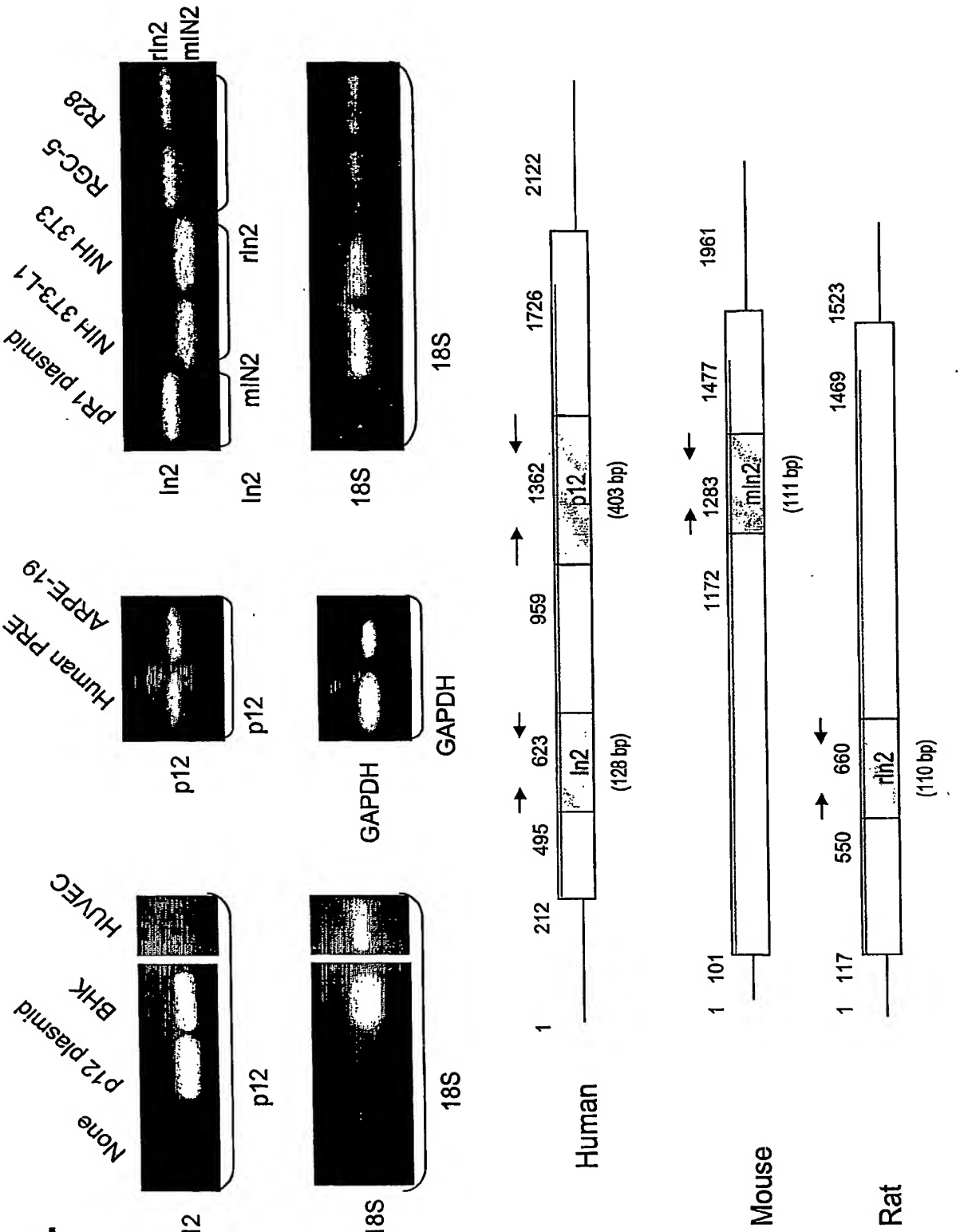
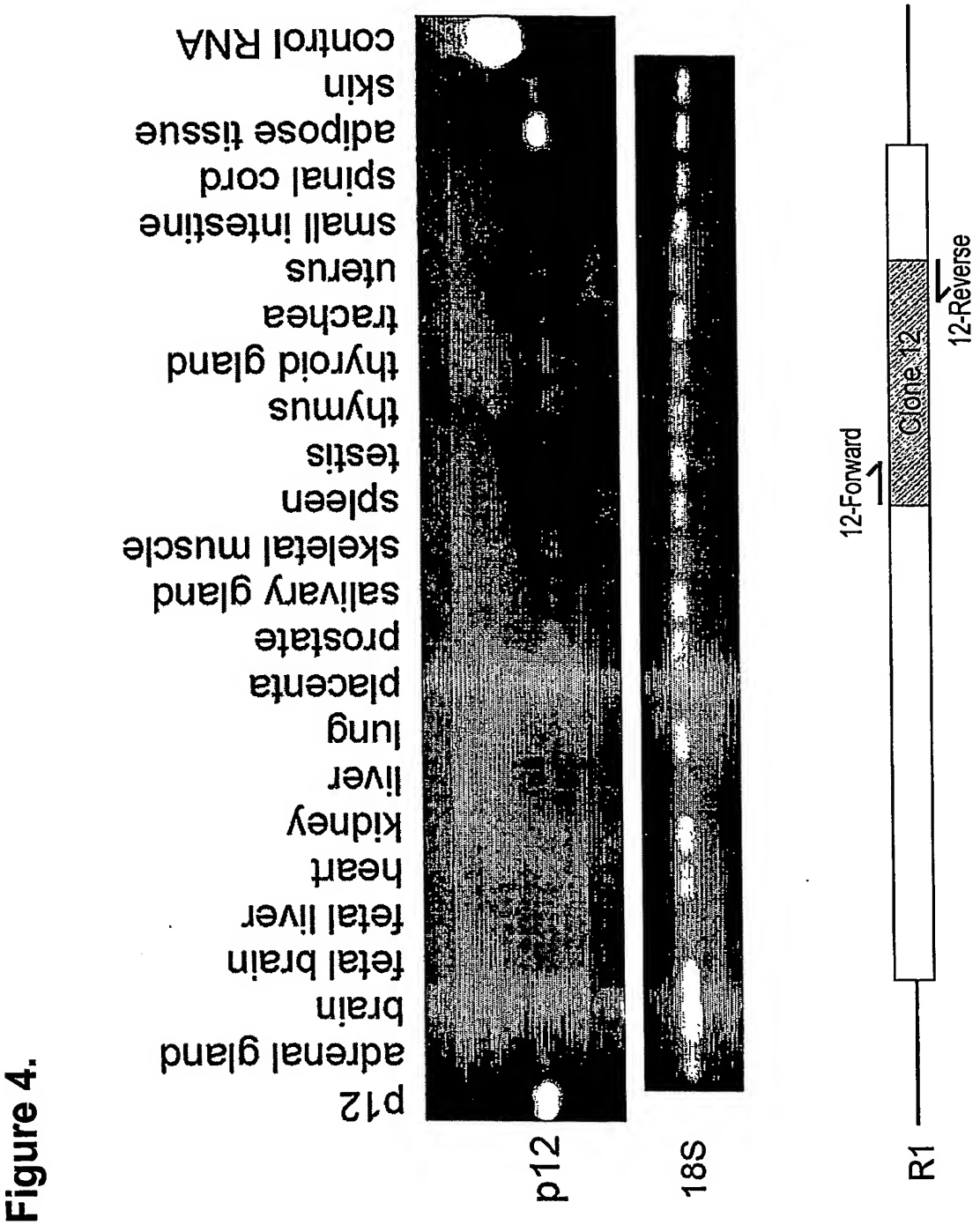


Figure 3.
Templates
Primers
Control primers
None
p12 plasmid
BHK
HUVEC
p12
18S
Human
Mouse
Rat
rIn2
mIN2
PR1 plasmid
NIH 3T3-L1
NIH 3T3
RGC-5
R28
Human PRE
ARPE-19
GAPDH
1
212
495
623
959
1362
1726
2122
(128 bp)
(403 bp)
1
101
1172
1283
1477
1961
(111 bp)
1
117
550
660
1469
1523
(110 bp)

Figure 4.


p12
adrenal gland
brain
fetal brain
fetal liver
heart
kidney
liver
lung
placenta
prostate
salivary gland
skeletal muscle
spleen
testis
thymus
thyroid gland
trachea
uterus
small intestine
spinal cord
adipose tissue
skin
control RNA
p12
18S
R1
12-Forward
Clone 12
12-Reverse

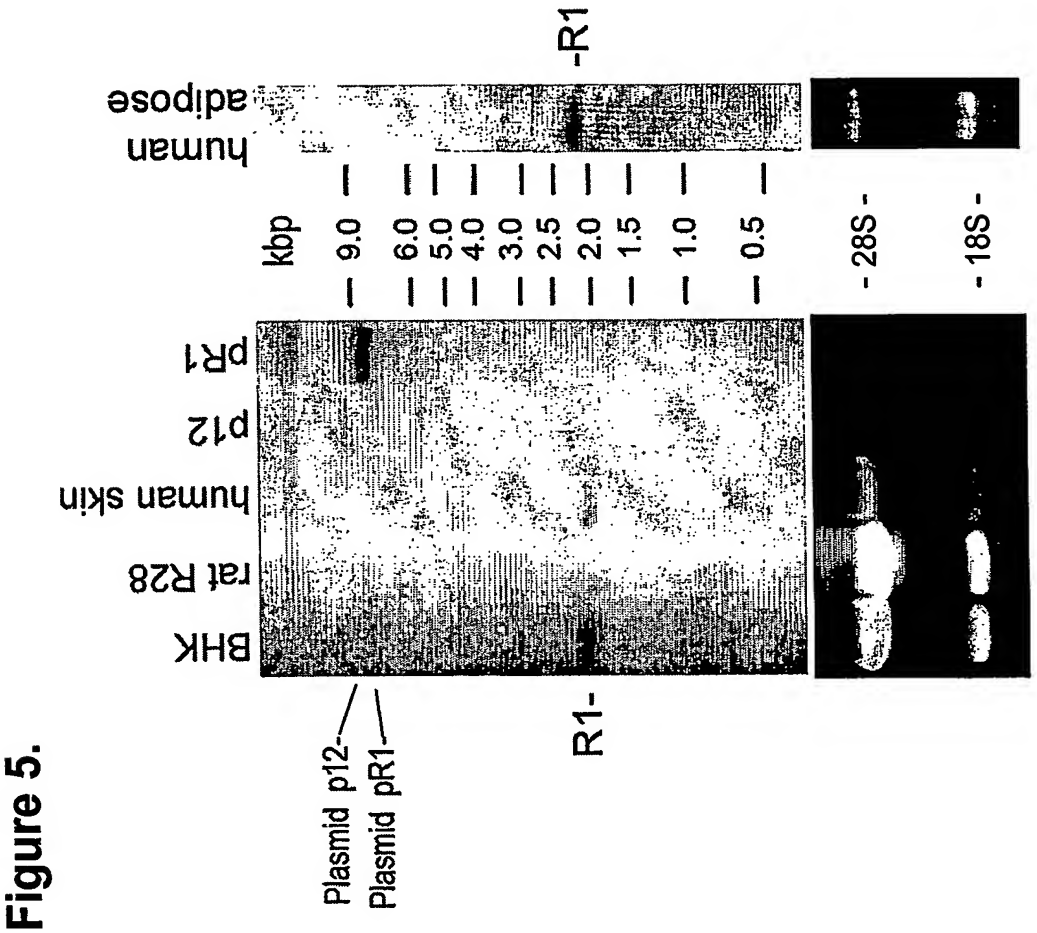
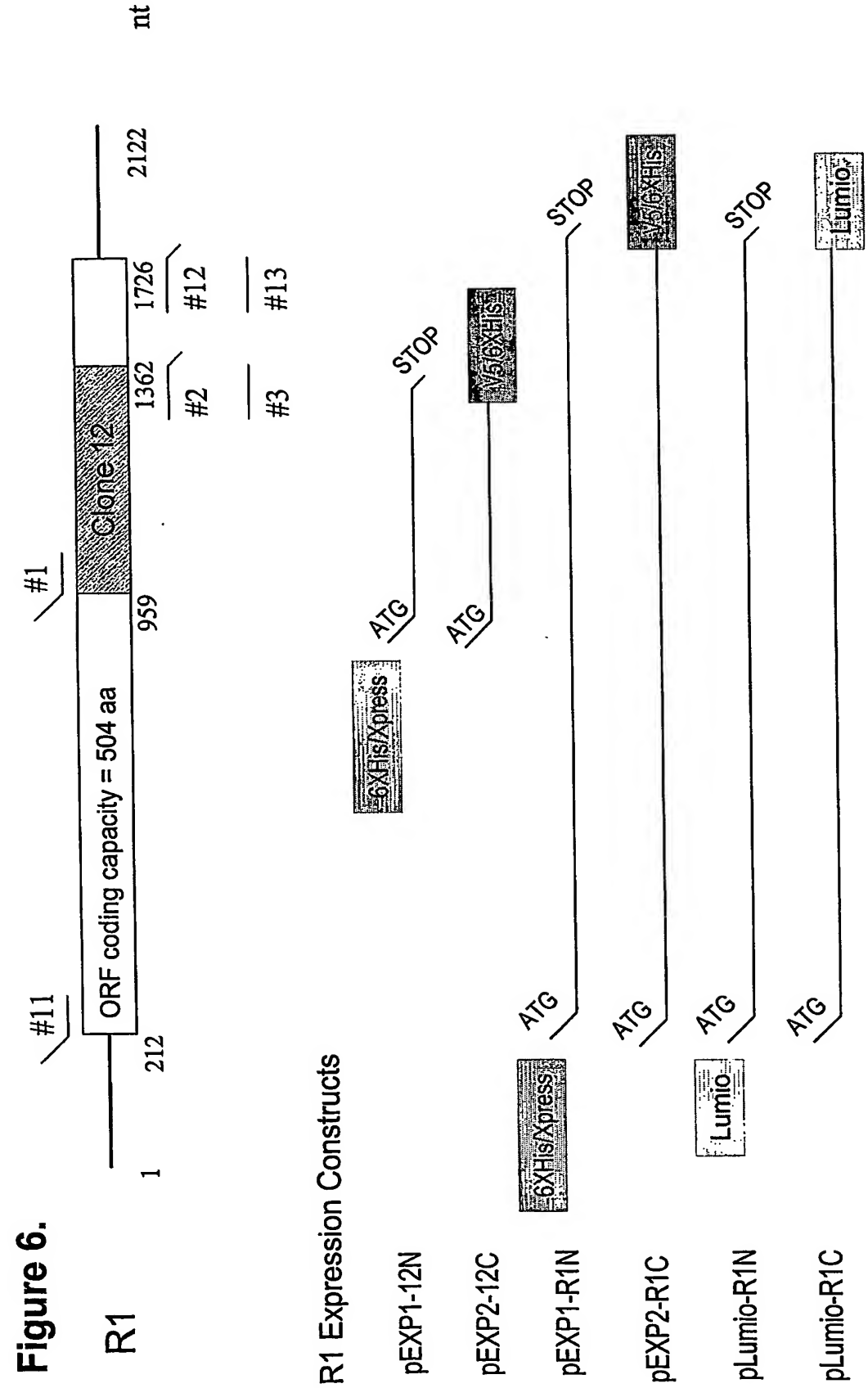

human adipose
-R1
kbp
9.0
6.0
5.0
4.0
3.0
2.5
2.0
1.5
1.0
0.5
- 28S -
- 18S -
BHK
rat R28
human skin
p12
pR1
Plasmid p12
Plasmid pR1
R1-

Figure 5.

Figure 6.


R1
#11
#1
ORF coding capacity = 504 aa
Clone 12
1
212
959
1362
1726
2122
nt
#2
#12
#3
#13
R1 Expression Constructs
pEXP1-12N
6XHis/Xpress
ATG
STOP
pEXP2-12C
V5/6XHis
ATG
STOP
pEXP1-R1N
6XHis/Xpress
ATG
STOP
pEXP2-R1C
V5/6XHis
ATG
pLumio-R1N
Lumio
ATG
STOP
pLumio-R1C
Lumio
ATG

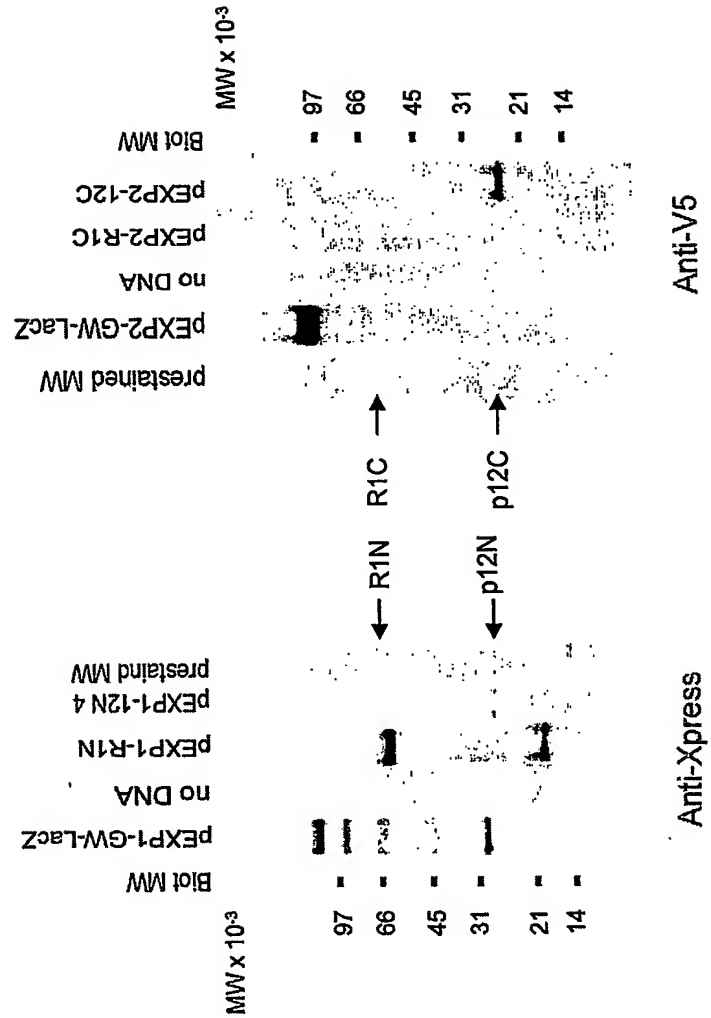

MW x 10-3
97
66
45
31
21
14
Blot MW
pEXP1-GW-LacZ
no DNA
pEXP1-R1N
pEXP1-12N 4
prestaind MW
R1N
p12N
Anti-Xpress
MW x 10-3
97
66
45
31
21
14
Blot MW
pEXP2-GW-LacZ
no DNA
pEXP2-R1C
pEXP2-12C
prestained MW
R1C
p12C
Anti-V5

Figure 7.

Figure 8.

A. p12

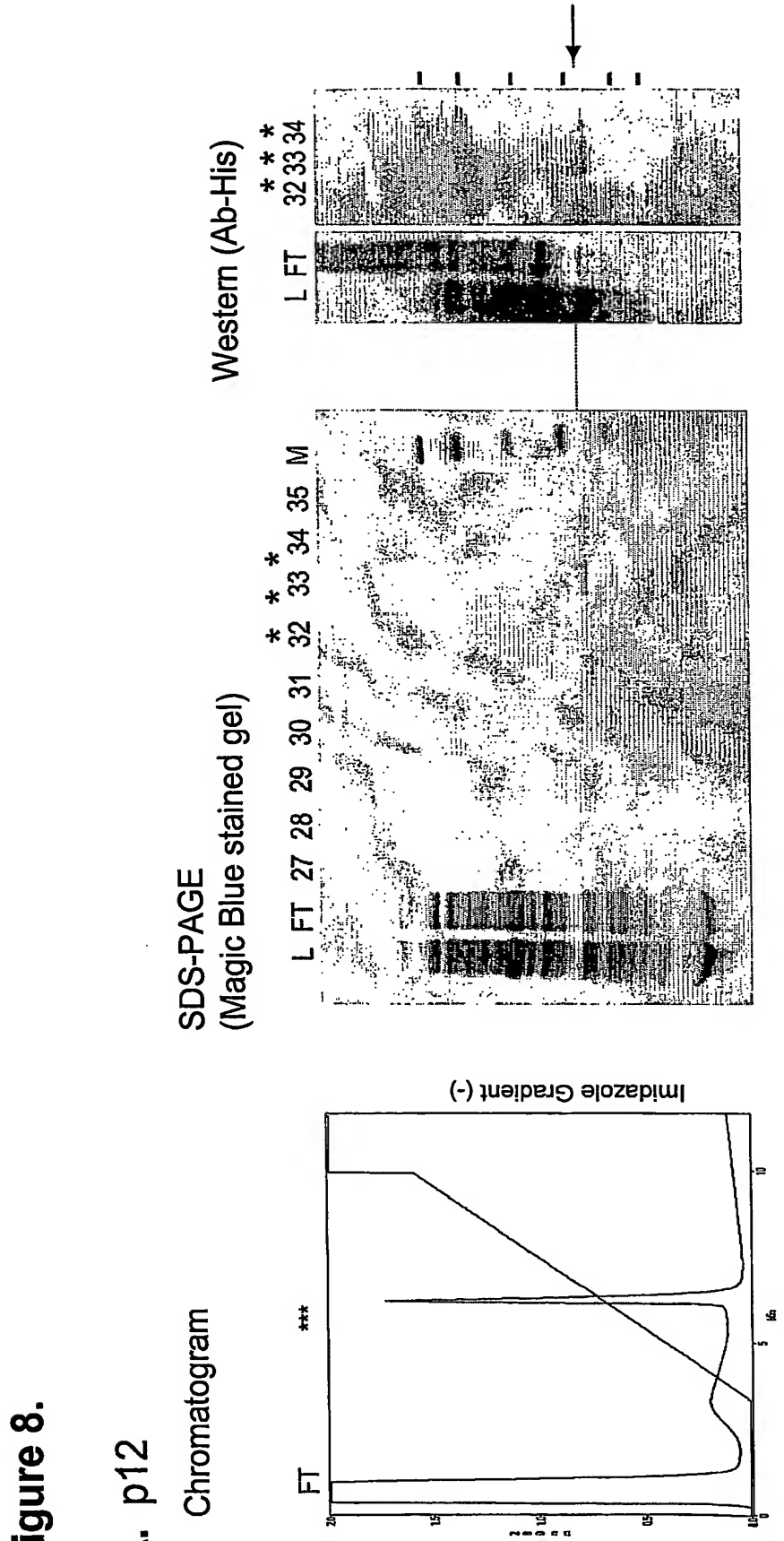
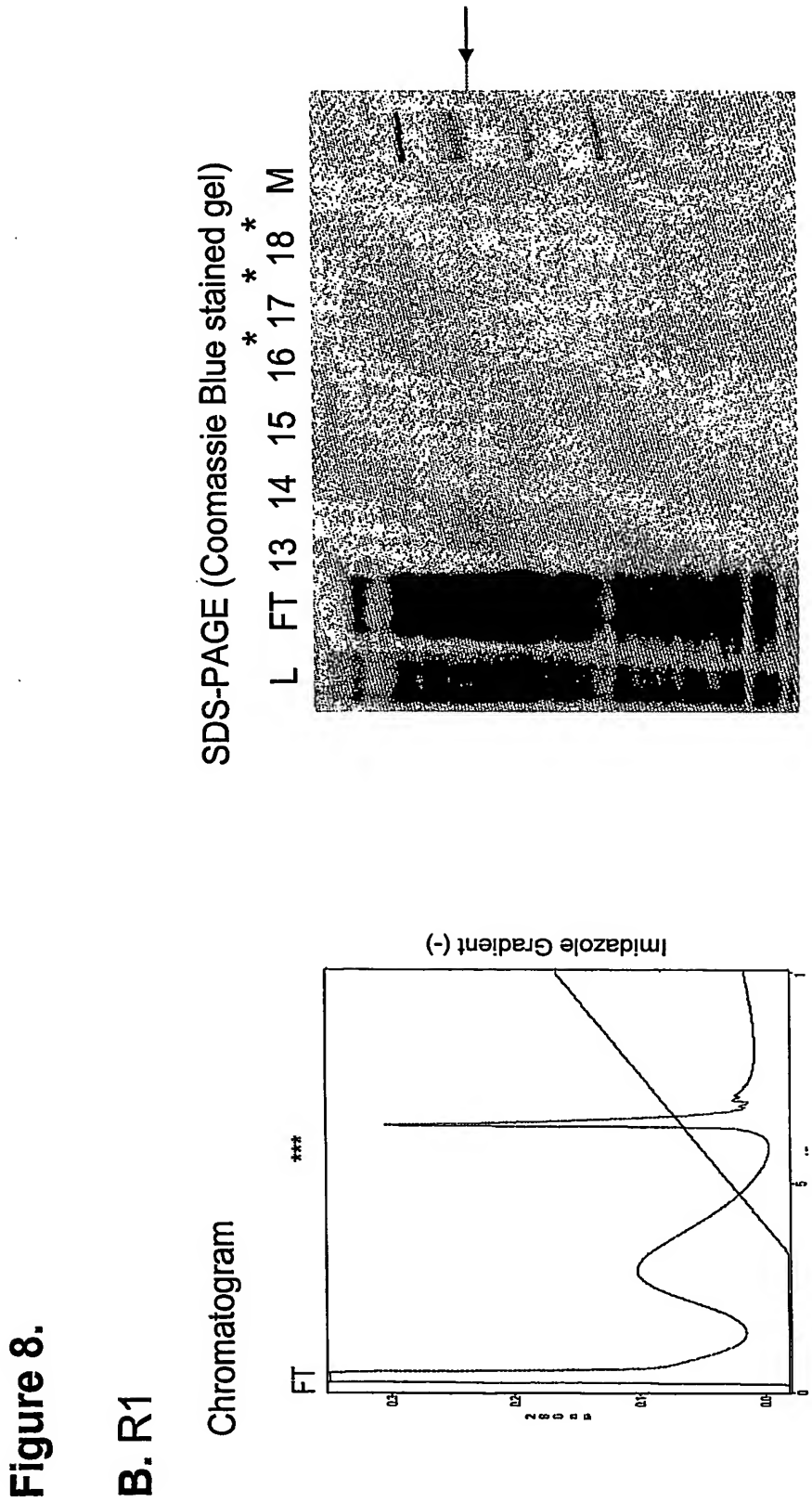


Figure 8.

B. R1


SDS-PAGE (Coomassie Blue stained gel)
L FT 13 14 15 16 17 18 M
* * *
Chromatogram
FT
***
Imidazole Gradient (-)

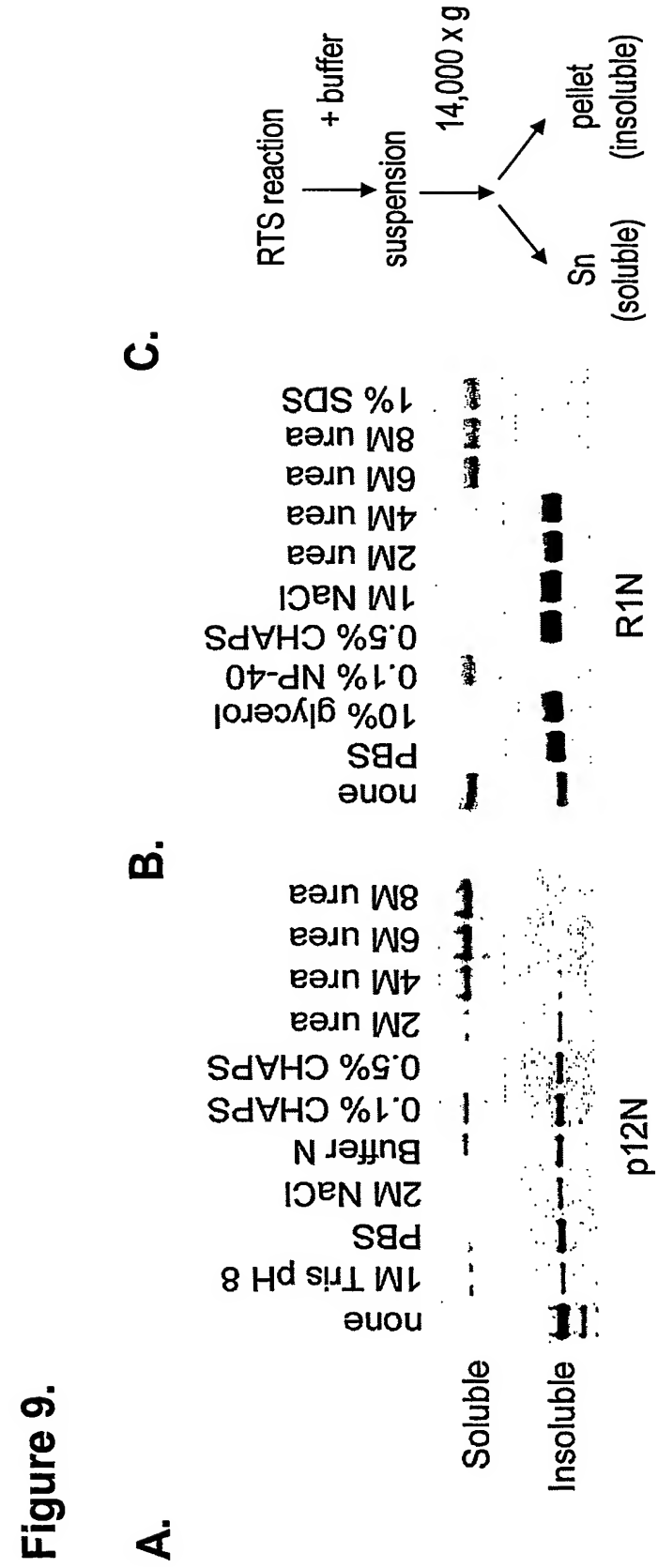

A.
Soluble
Insoluble
p12N
none
1M Tris pH 8
PBS
2M NaCl
Buffer N
0.1% CHAPS
0.5% CHAPS
2M urea
4M urea
6M urea
8M urea
B.
R1N
none
PBS
10% glycerol
0.1% NP-40
0.5% CHAPS
1M NaCl
2M urea
4M urea
6M urea
8M urea
1% SDS
C.
RTS reaction
+ buffer
suspension
14,000 x g
pellet
(insoluble)
Sn
(soluble)

Figure 9.

Figure 10.

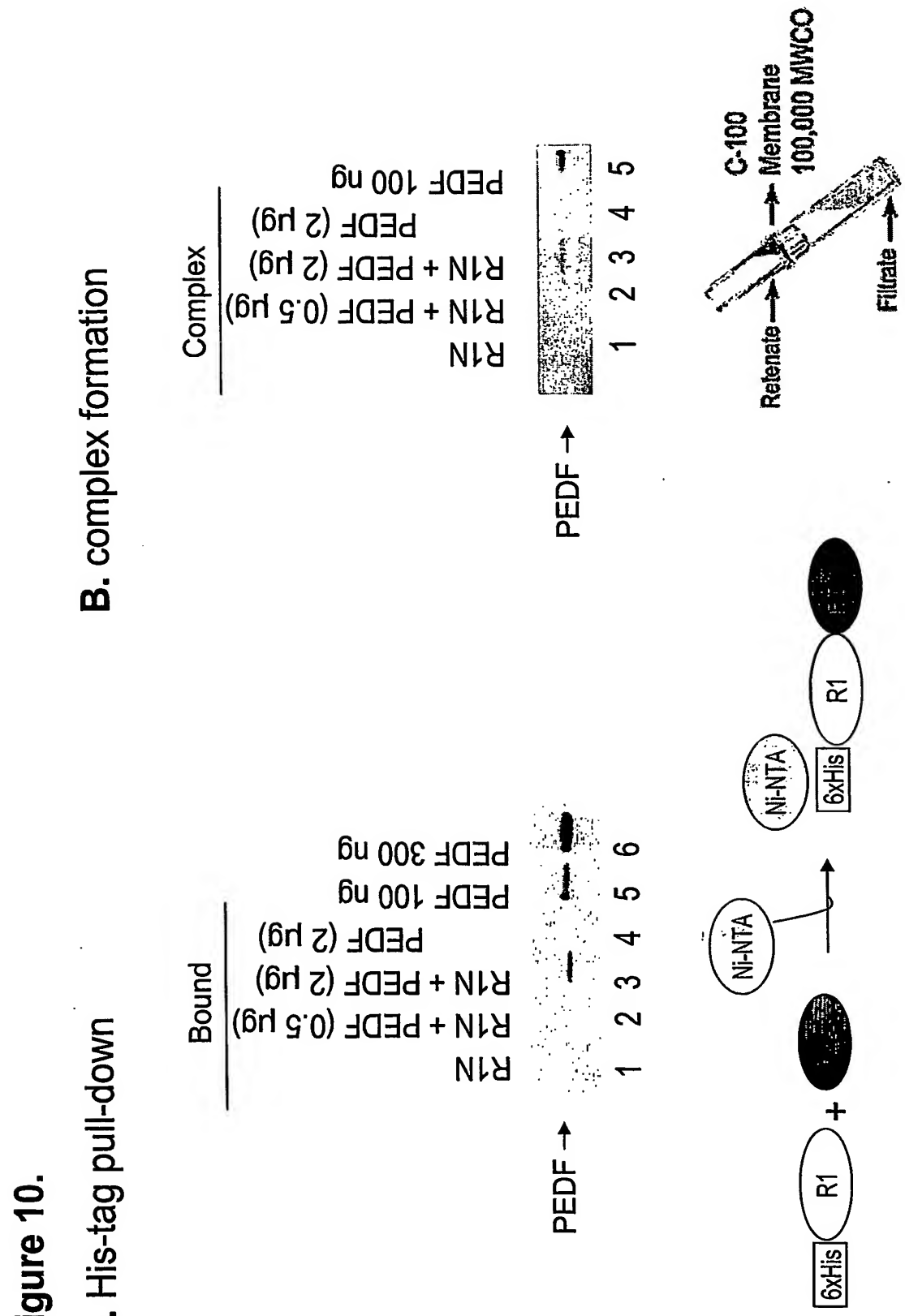

A. His-tag pull-down
B. complex formation
Bound
R1N
R1N + PEDF (0.5 µg)
R1N + PEDF (2 µg)
PEDF (2 µg)
PEDF 100 ng
PEDF 300 ng
1 2 3 4 5 6
PEDF →
Complex
R1N
R1N + PEDF (0.5 µg)
R1N + PEDF (2 µg)
PEDF (2 µg)
PEDF 100 ng
1 2 3 4 5
PEDF →
C-100
Membrane
100,000 MWCO
Retenate
Filtrate
6xHis
R1
+
Ni-NTA
Ni-NTA
6xHis
R1

Figure 11.

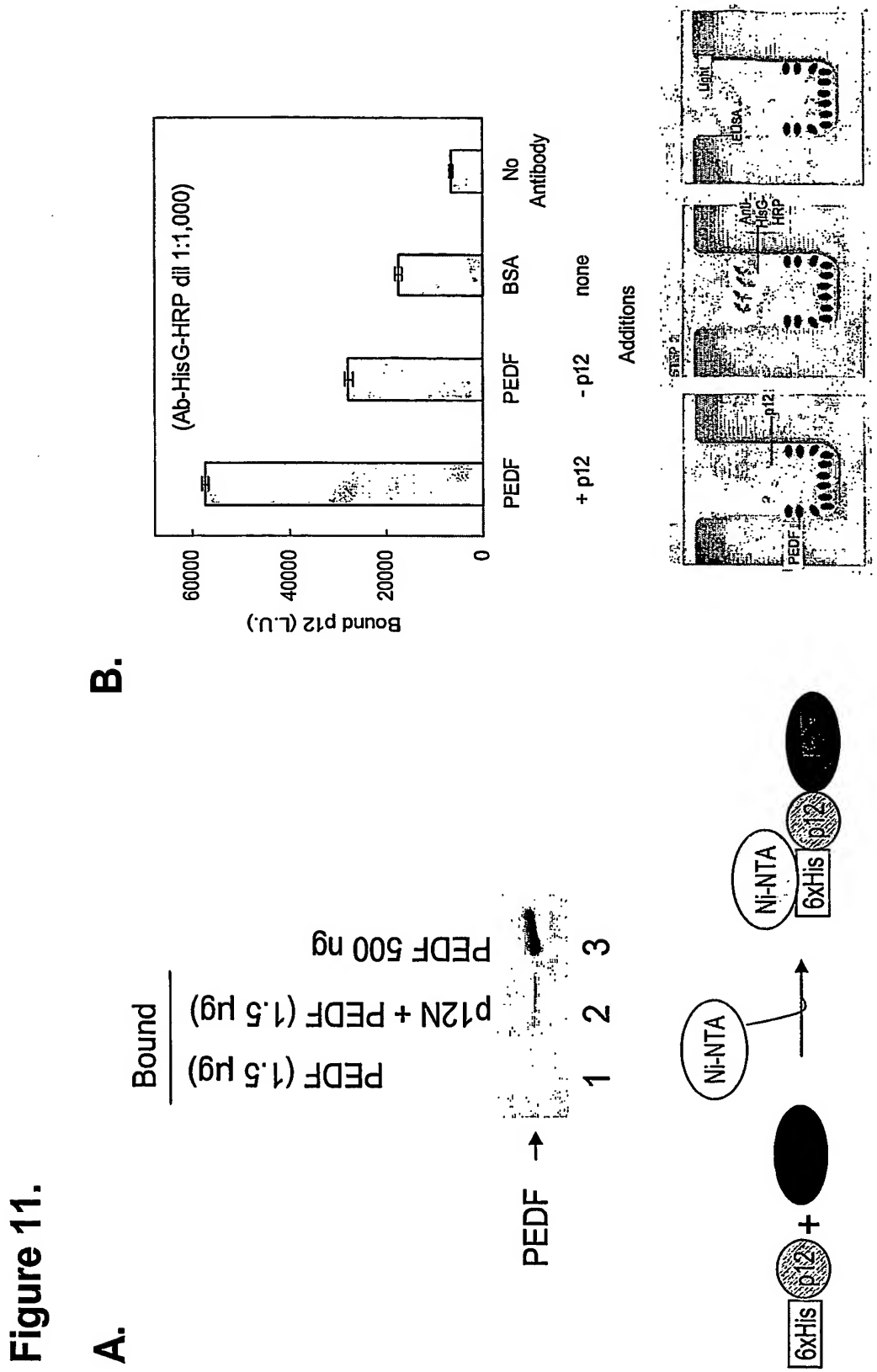

A.
PEDF
1 2 3
Bound
PEDF (1.5 µg)
p12N + PEDF (1.5 µg)
PEDF 500 ng
6xHis
p12
Ni-NTA
Ni-NTA
6xHis
p12
B.
Bound p12 (L.U.)
60000
40000
20000
0
(Ab-HisG-HRP dil 1:1,000)
PEDF
PEDF
BSA
No Antibody
+ p12
- p12
none
Additions
STEP 1
STEP 2
PEDF
p12
Anti-HisG-HRP
Light
BSA

Figure 12.

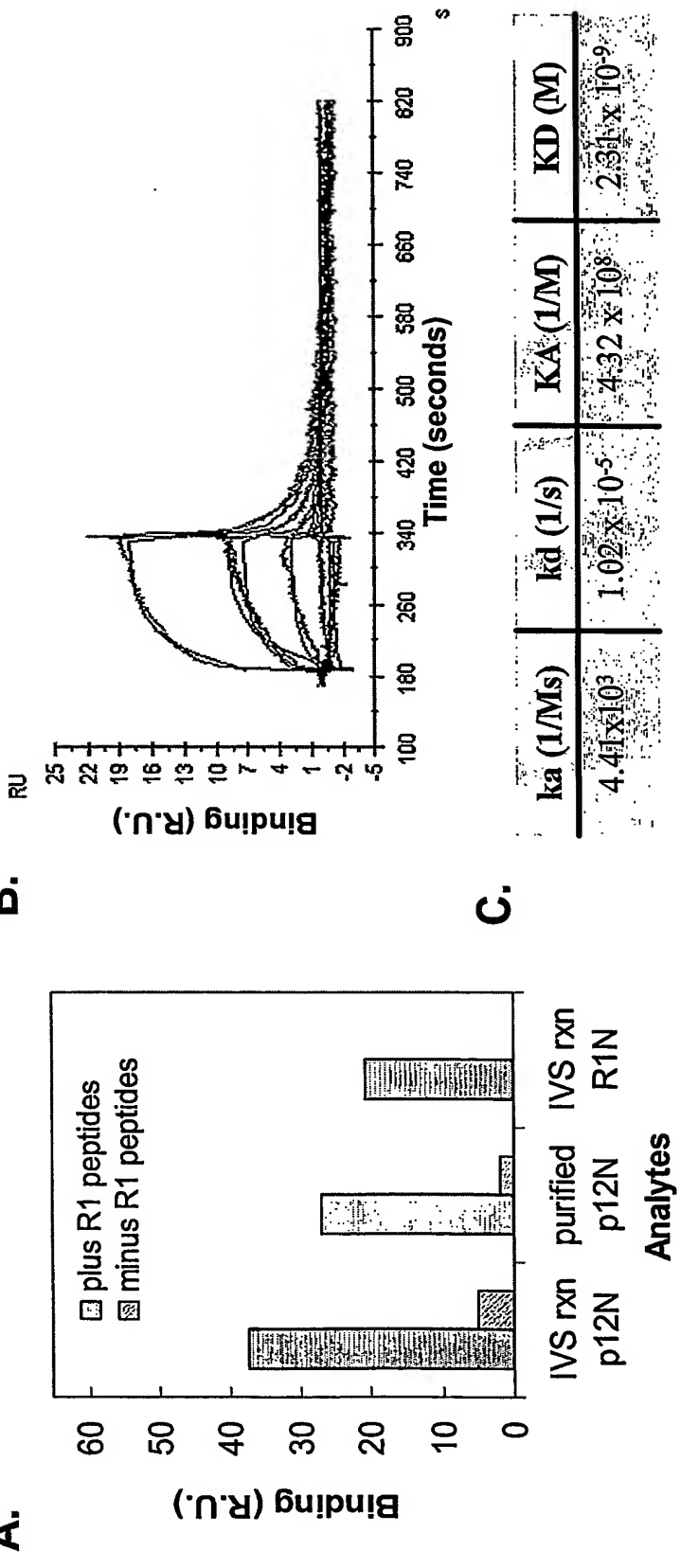

A.
Binding (R.U.)
plus R1 peptides
minus R1 peptides
IVS rxn p12N
purified p12N
IVS rxn R1N
Analytes
B.
RU
Binding (R.U.)
Time (seconds)
s
C.
ka (1/Ms)
kd (1/s)
KA (1/M)
KD (M)
4.41x10^3
1.02 x 10^-5
4.32 x 10^8
2.31 x 10^-9

Figure 13.

A.

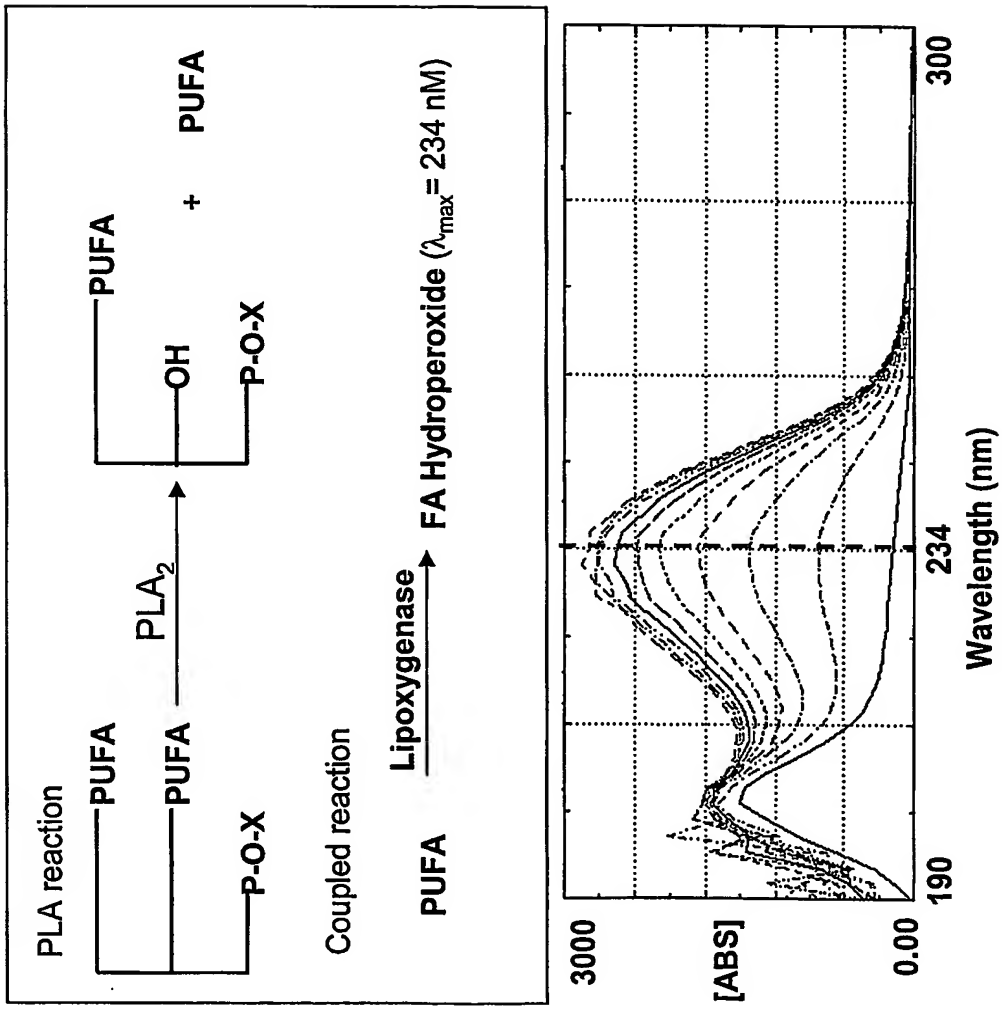
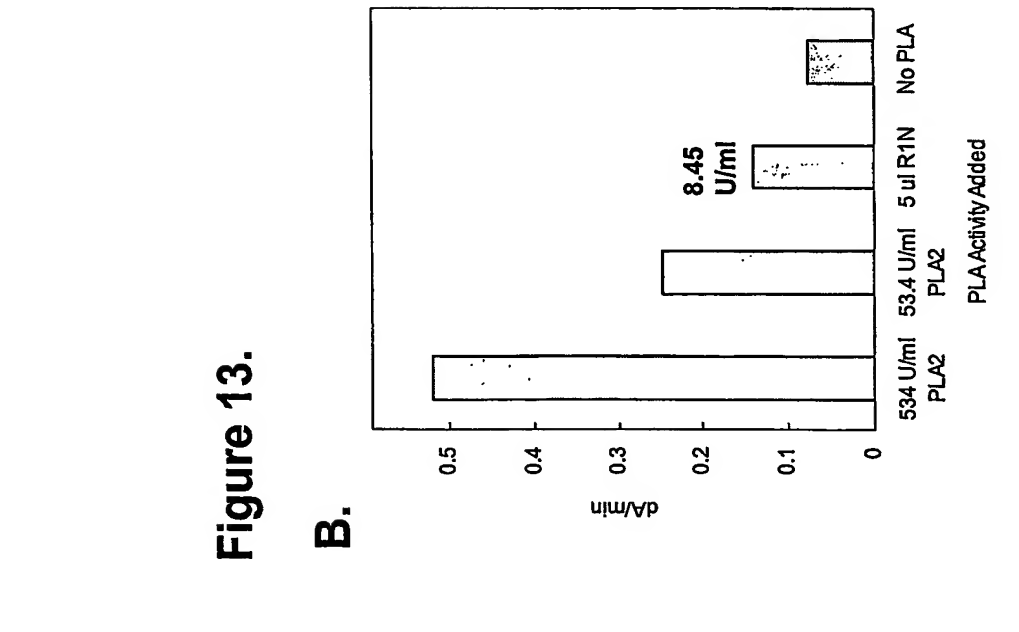

PLA reaction
PUFA
PUFA
P-O-X
PLA2
OH
PUFA
P-O-X
+
PUFA
Coupled reaction
Lipoxygenase
PUFA
FA Hydroperoxide (λmax= 234 nM)
3000
[ABS]
0.00
190
234
300
Wavelength (nm)

Figure 13.


B.
dA/min
0.5
0.4
0.3
0.2
0.1
0
534 U/ml PLA2
53.4 U/ml PLA2
5 ul R1N
No PLA
8.45 U/ml
PLA Activity Added
C.
PLA activity (dAbs234 nm/min)
0.16
0.12
0.08
0.04
0
pH 7.5
pH 8.5
Tris
pH 8.5
pH 9.5
Borate
Reaction buffer conditions

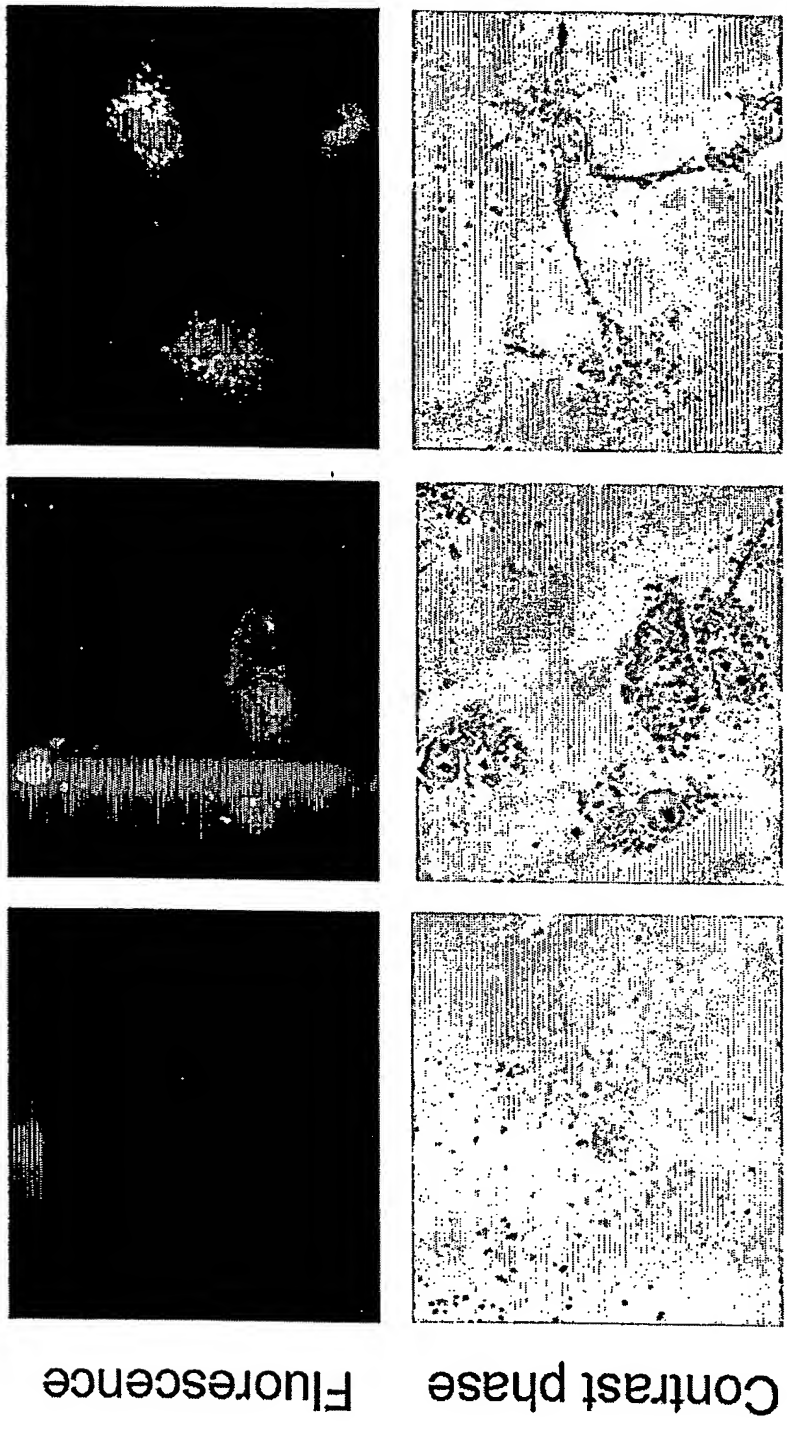


Figure 14.
A. COS-7 cells

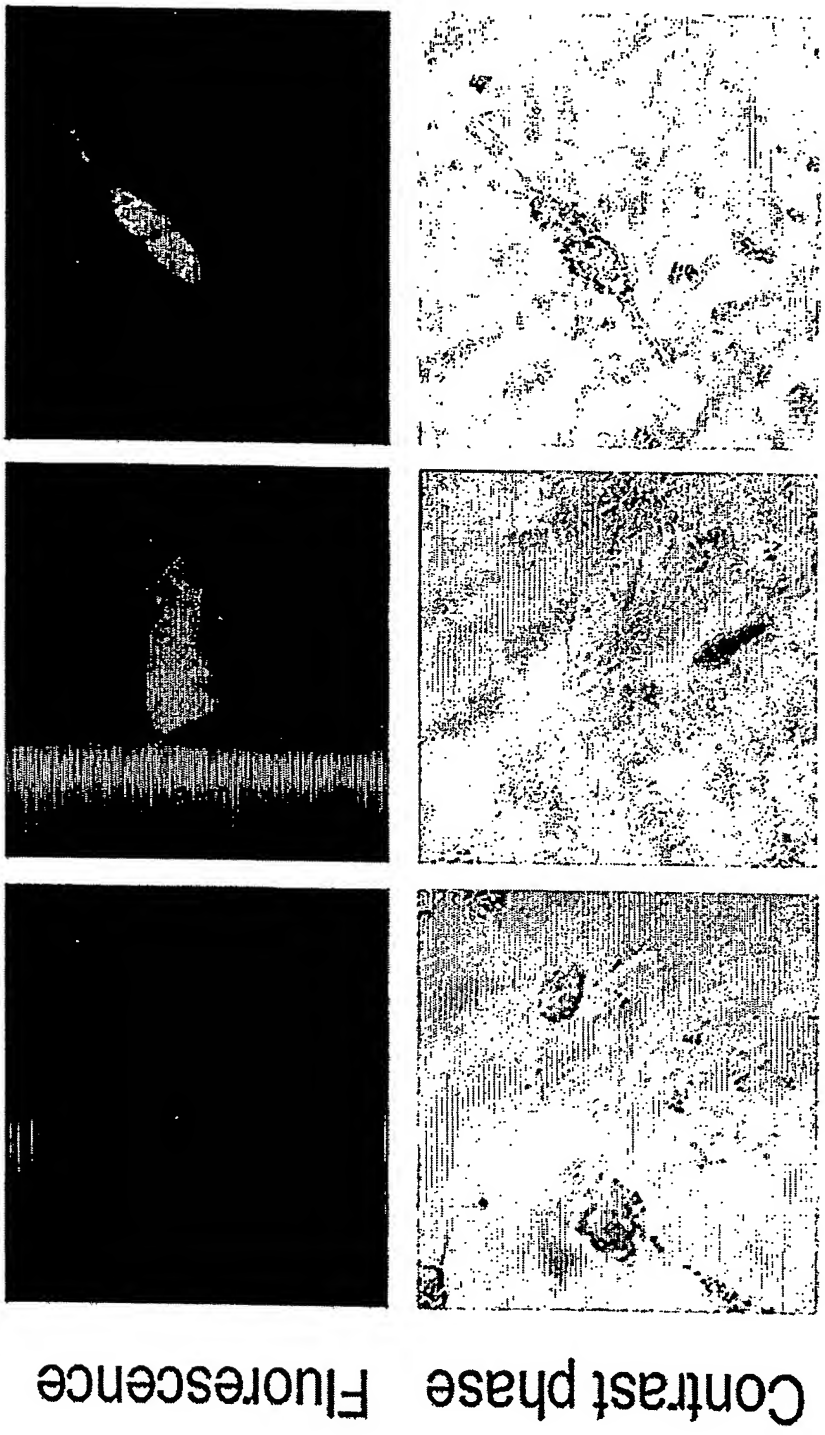
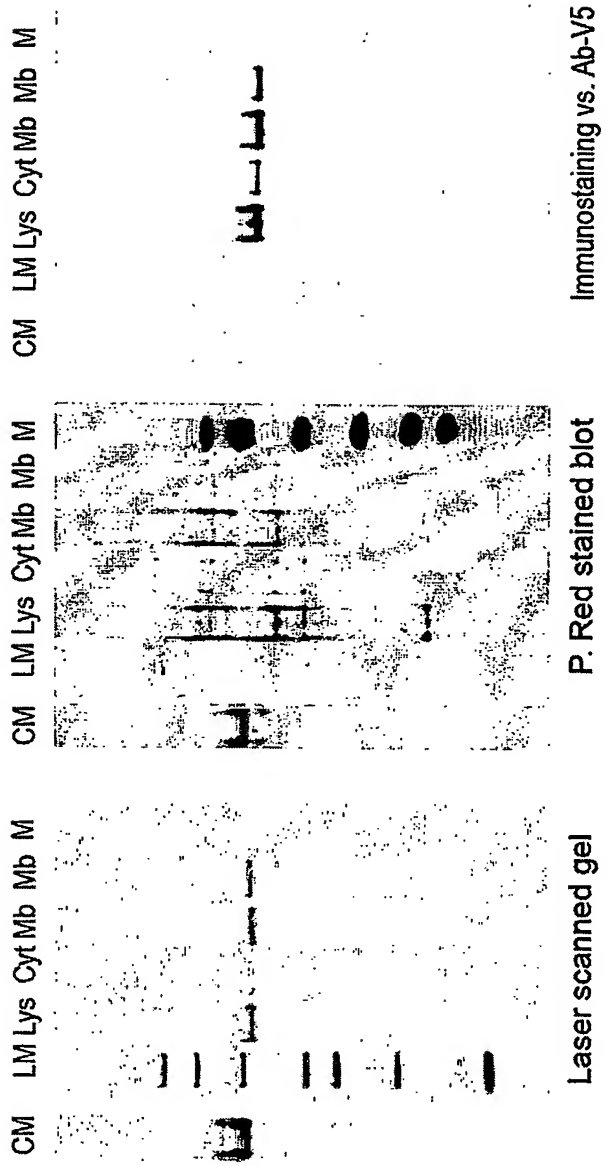


Figure 14.
B. Retinal ganglion RGC-5 cells

Figure 15.



LM= Lumio markers, M = MW standards CM, conditioned media; Lys, Lysate; Cyt, cytosolic; Mb, membrane fractions

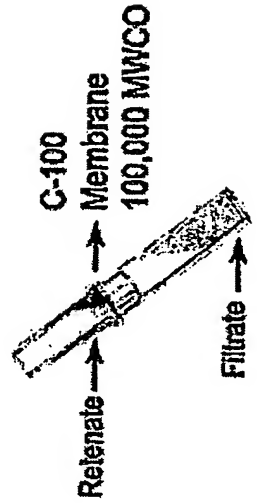

C-100
Membrane
100,000 MWCO
Retenate
Filtrate

Figure 16.

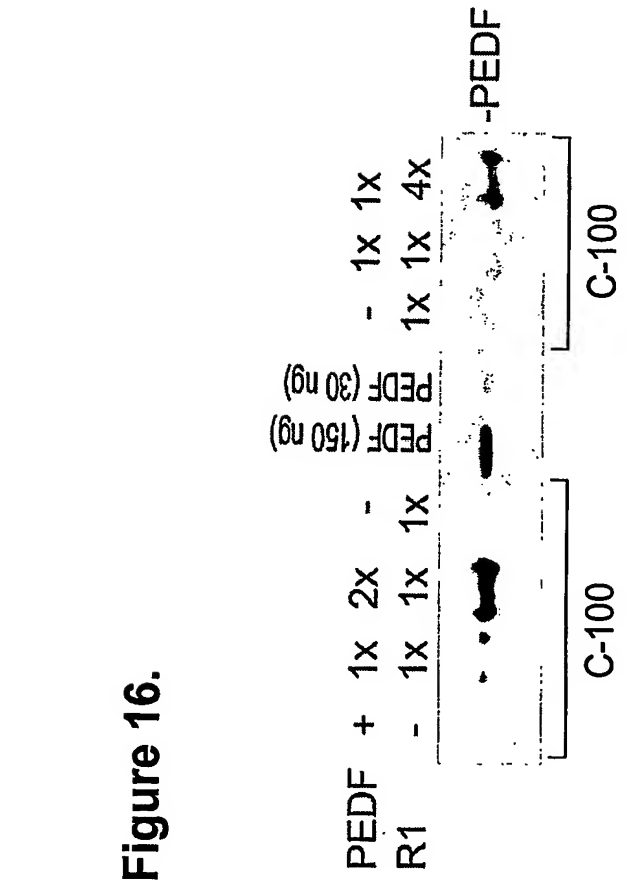
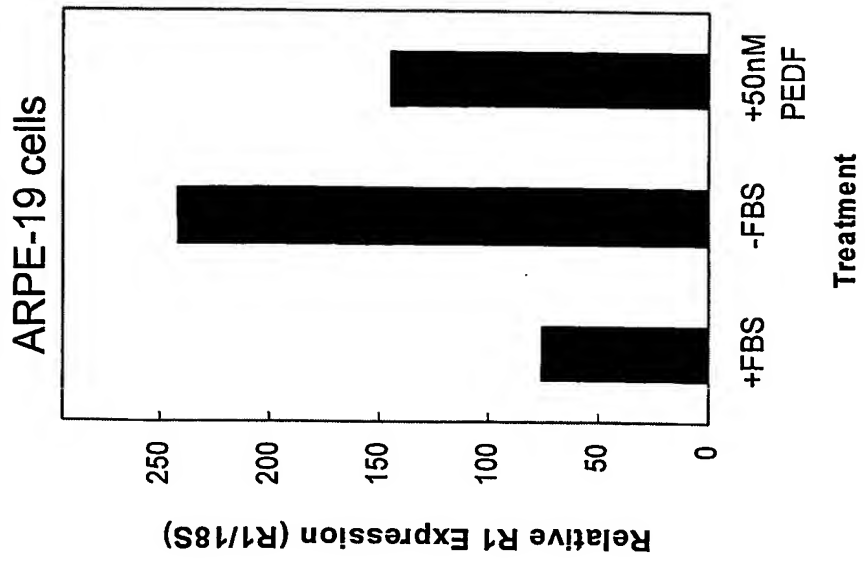

PEDF + 1x 2x -
R1 - 1x 1x 1x
PEDF (150 ng)
PEDF (30 ng)
- 1x 1x
1x 1x 4x
-PEDF
C-100
C-100

Figure 17.

A.


ARPE-19 cells
Relative R1 Expression (R1/18S)
250
200
150
100
50
0
+FBS
-FBS
+50nM PEDF
Treatment

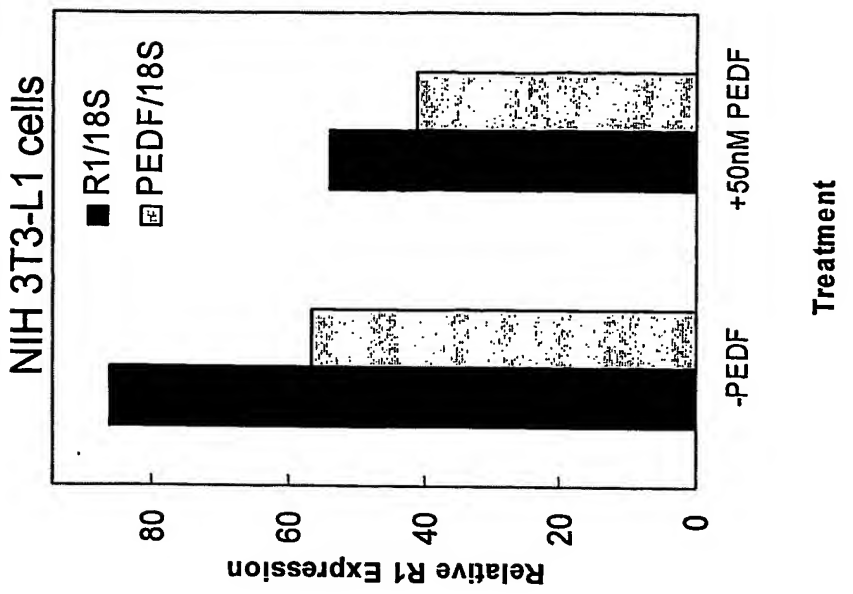

NIH 3T3-L1 cells
R1/18S
PEDF/18S
Relative R1 Expression
80
60
40
20
0
-PEDF
+50nM PEDF
Treatment

Figure 17.

B.

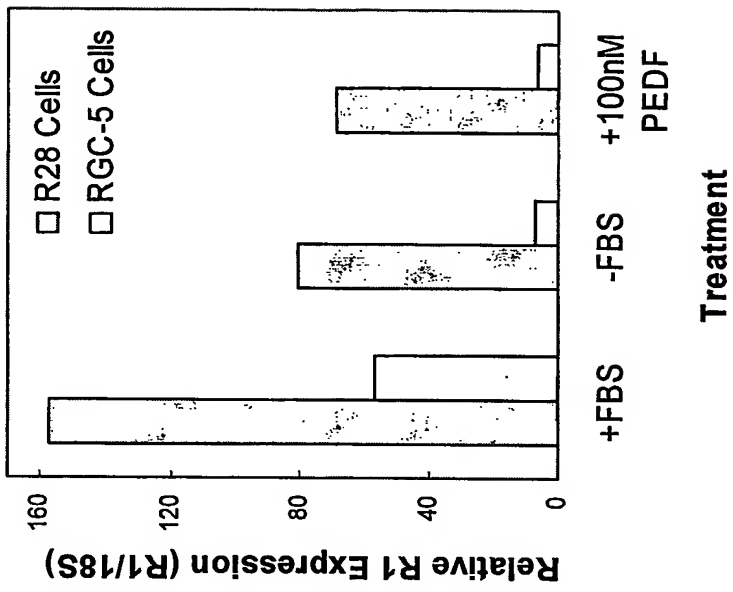

R28 Cells
RGC-5 Cells
Relative R1 Expression (R1/18S)
160
120
80
40
0
+FBS
-FBS
+100nM PEDF
Treatment

Figure 17.

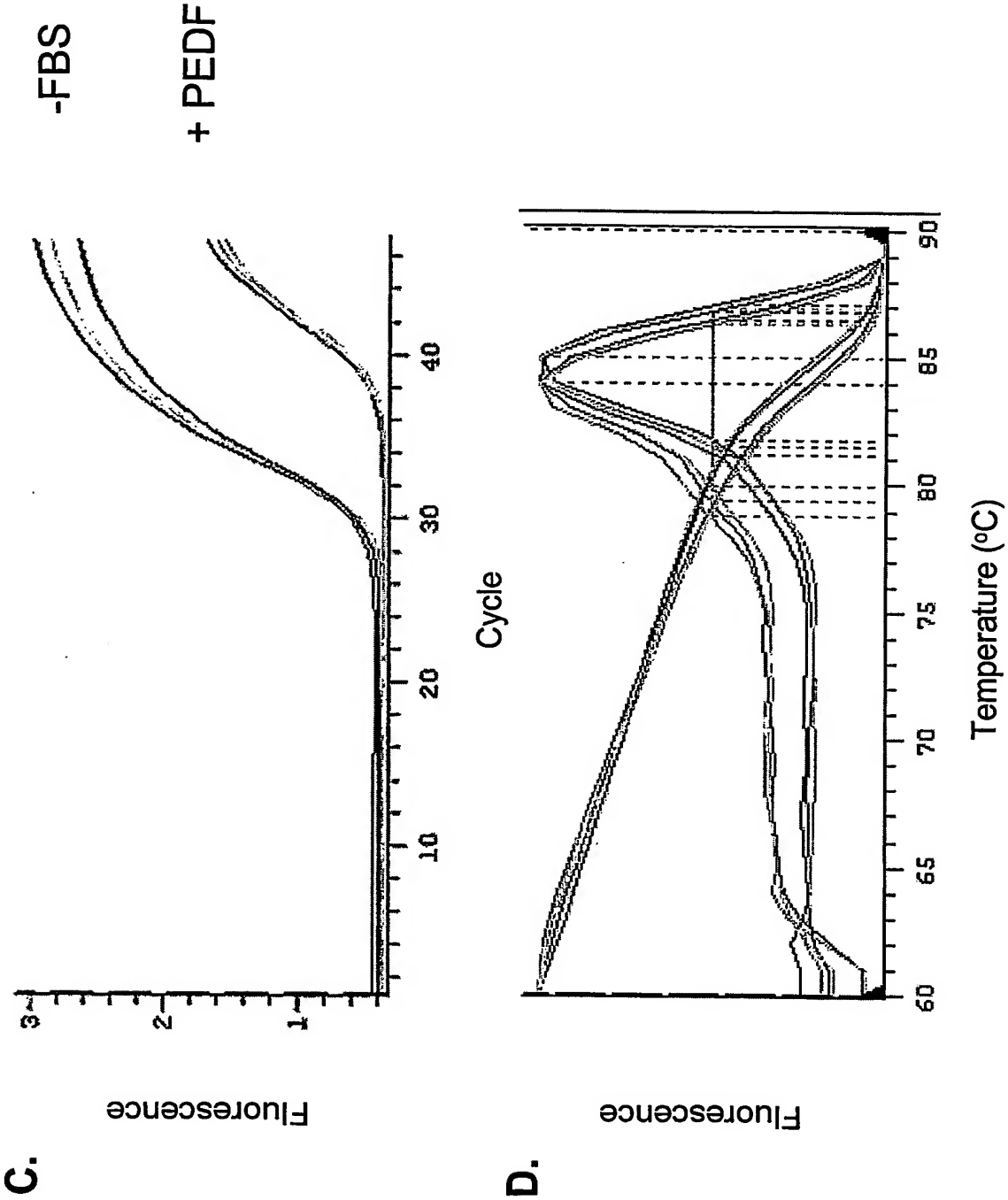

C.
-FBS
+ PEDF
Fluorescence
3
2
1
10
20
30
40
D.
Fluorescence
Cycle
Temperature (°C)
60
65
70
75
80
85
90

Figure 18.

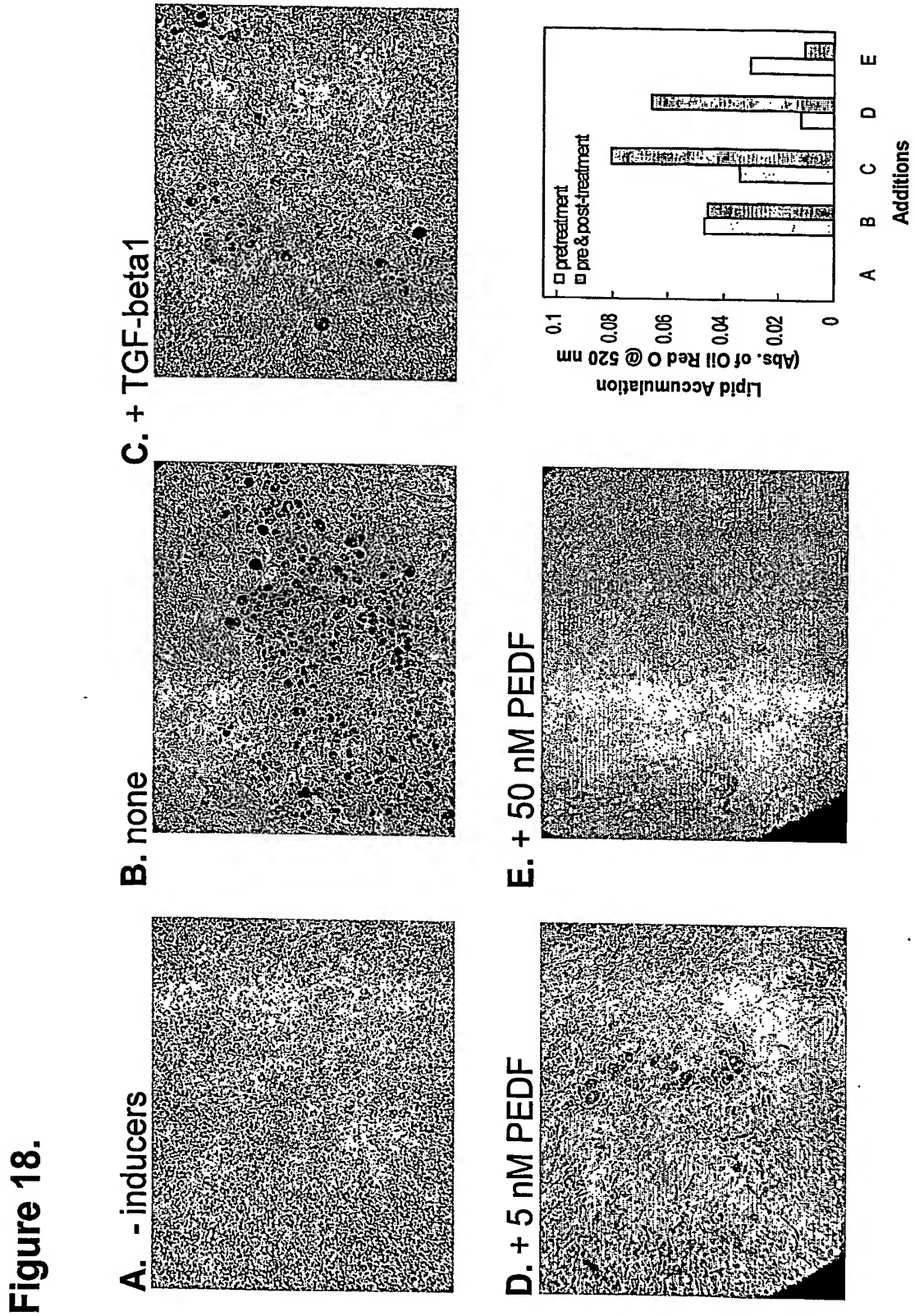

A. - inducers
B. none
C. + TGF-beta1
D. + 5 nM PEDF
E. + 50 nM PEDF
Lipid Accumulation
(Abs. of Oil Red O @ 520 nm
0.1
0.08
0.06
0.04
0.02
0
pretreatment
pre & post-treatment
A B C D E
Additions

Figure 19.

CLUSTAL W (1.82) multiple sequence alignment

```
gi|26327465|dbj|BAC27476.1|     MFPRETKWNISFAGCGFLGVYHIGVASCLREHAPFLVANATHIYGASAGA 50
gi|34861242|ref|XP_341961.1|    MFPRETKWNISFAGCGFLGVYHIGVASCLREHAPFLVANATHIYGASAGA 50
gi|16878147|gb|AAH17280.1|      MFPREKTWNISFAGCGFLGVYYVGVASCLREHAPFLVANATHIYGASAGA 50
                                *****..**************::**************************** 

gi|26327465|dbj|BAC27476.1|     LTATALVTGACLGEAGANIIEVSKEARKRFLGPLHPSFNLVKTIRGCLLK 100
gi|34861242|ref|XP_341961.1|    LTATALVTGACLGEAGANIIEVSKEARKRFLGPLHPSFNLVKTIRGCLLK 100
gi|16878147|gb|AAH17280.1|      LTATALVTGVCLGEAGAKFIEVSKEARKRFLGPLHPSFNLVKIIRSFLLK 100
                                *********.*******::************************ **. *** 

gi|26327465|dbj|BAC27476.1|     TLPADCHERANGRLGISLTRVSDGENVIISHFSSKDELIQANVCSTFIPV 150
gi|34861242|ref|XP_341961.1|    TLPADCHTRASGRLGISLTRVSDGENVIISHFSSKDELIQANVCSTFIPV 150
gi|16878147|gb|AAH17280.1|      VLPADSHEHASGRLGISLTRVSDGENVIISHFNSKDELIQANVCSGFIPV 150
                                .****.* :*.**********************.************ **** 

gi|26327465|dbj|BAC27476.1|     YCGLIPPTLQGVRYVDGGISDNLPLYELKNTITVSPFSGESDICPQDSST 200
gi|34861242|ref|XP_341961.1|    YCGLIPPTLQGVRYVDGGISDNLPLYELKNTITVSPFSGESDICPQDSST 200
gi|16878147|gb|AAH17280.1|      YCGLIPPSLQGVRYVDGGISDNLPLYELKNTITVSPFSGESDICPQDSST 200
                                *******:****************************************** 

gi|26327465|dbj|BAC27476.1|     NIHELRVTNTSIQFNLRNLYRLSKALFPPEPMVLREMCKQGYRDGLRFLR 250
gi|34861242|ref|XP_341961.1|    NIHELRITNTSIQFNLRNLYRLSKALFPPEPMVLREMCKQGYRDGLRFLR 250
gi|16878147|gb|AAH17280.1|      NIHELRVTNTSIQFNLRNLYRLSKALFPPEPLVLREMCKQGYRDGLRFLQ 250
                                ******:************************:*****************: 

gi|26327465|dbj|BAC27476.1|     RNGLLNQPNPLLALPPVVPQEEDAEEAAVVEERAGEEDQLQPYRKDRILE 300
gi|34861242|ref|XP_341961.1|    RNGLLNQPNPLLALPPVVPQEEDAEEAAVTEERTGGED--------RILE 292
gi|16878147|gb|AAH17280.1|      RNGLLNRPNPLLALPPARPHGPEDKDQAVESAQAEDYSQLP--GEDHILE 298
                                ******:*********. *:  : :: ** . ::   .        :*** 

gi|26327465|dbj|BAC27476.1|     HLPARLNEALLEACVEPKDLMTTLSNMLPVRLATAMMVPYTLPLESAVSF 350
gi|34861242|ref|XP_341961.1|    HLPARLNEALLEACVEPKDLMTTLSNMLPVRLATAMMVPYTLPLESAVSF 342
gi|16878147|gb|AAH17280.1|      HLPARLNEALLEACVEPTDLLTTLSNMLPVRLATAMMVPYTLPLESALSF 348
                                ****************.**:***************************:** 

gi|26327465|dbj|BAC27476.1|     TIRLLEWLPDVPEDIRWMKEQTGSICQYLVMRAKRKLGDHLPSRLSEQVE 400
gi|34861242|ref|XP_341961.1|    TIRLLEWLPDVPEDIRWMKEQTGSICQYLVMRAKRKLGDHLPSRLSEQVE 392
gi|16878147|gb|AAH17280.1|      TIRLLEWLPDVPEDIRWMKEQTGSICQYLVMRAKRKLGRHLPSRLPEQVE 398
                                ************************************ *******.**** 

gi|26327465|dbj|BAC27476.1|     LRRAQSLPSVPLSCATYSEALPNWVRNNLSLGDALAKWEECQRQLLLGLF 450
gi|34861242|ref|XP_341961.1|    LRRAQSLPSVPLSCATYSEALPNWVRNNLSLGDALAKWEECQRQLLLGLF 442
gi|16878147|gb|AAH17280.1|      LRRVQSLPSVPLSCAAYREALPGWMRNNLSLGDALAKWEECQRQLLLGLF 448
                                ***.***********:* ****.*:************************** 

gi|26327465|dbj|BAC27476.1|     CTNVAFPPDALRMRAPAS--PTAADPATPQDPPGLPPC------------ 486
gi|34861242|ref|XP_341961.1|    CTNVAFPPDALRMRAPAS--PTATDPATPQDPSGLPPC------------ 478
gi|16878147|gb|AAH17280.1|      CTNVAFPPEALRMRAPADPAPAPADPASPQHQLAGPAPLLSTPAPEARPV 498
                                ********:********.  *:.:***:**.  . *. 

gi|26327465|dbj|BAC27476.1|     ------
gi|34861242|ref|XP_341961.1|    ------
gi|16878147|gb|AAH17280.1|      IGALGL 504
```

Figure 20. (1 of 4)

CLUSTAL W (1.82) multiple sequence alignment

```
gi|26327464|dbj|AK031609.1|     ----------------------------------------------------
gi|34861241|ref|XM_341960.1|    ----------------------------------------------------
gi|16878146|gb|BC017280.1|BC01  GGCACGAGGGCGGCCCCAGTCAGACGCAGGCAGCCCCAAAGCCTGAACAG 50

gi|26327464|dbj|AK031609.1|     ----------------------------------------------------
gi|34861241|ref|XM_341960.1|    ----------------------------------------------------
gi|16878146|gb|BC017280.1|BC01  GCAGGGCCAGACCCAGCTTCTTCGCCTCCGCCAGCGGGGACCCCGAGCTA 100

gi|26327464|dbj|AK031609.1|     ----------------------------------------------------
gi|34861241|ref|XM_341960.1|    ----------------TCCTCTGCCTCCCGGCACAGCGTCTCCGCCTCCG 34
gi|16878146|gb|BC017280.1|BC01  GAGCCGCAGCGGGACCTGCCCGGCCCCCGGCTCCAGCGAGCGAGCGGCGA 150

gi|26327464|dbj|AK031609.1|     -----GGAGACCCCAAGGTATCGA-GACTGCGGGACCCACTGCCCGCAGG 44
gi|34861241|ref|XM_341960.1|    CCGGCGGGGACCCCAGGTTATCAA-GACTGCGGGACCCACTGCCCGCAGG 83
gi|16878146|gb|BC017280.1|BC01  GCAGGCGGCTCACAGAGGCCTGGCCGCCCACGGAACCCGGGGCCCGGCGG 200
                                      *   * *   *   *    * *  *** ****   *****  **

gi|26327464|dbj|AK031609.1|     ACATCGAGTCACGATGTTCCCGAGGGAGACCAAGTGGAACATCTCATTCG 94
gi|34861241|ref|XM_341960.1|    ACGTCTAATCACGATGTTCCCAAGGGAGACCAAGTGGAACATCTCGTTCG 133
gi|16878146|gb|BC017280.1|BC01  CCGCC--GCCGCGATGTTTCCCCGCGAGAAGACGTGGAACATCTCGTTCG 248
                                 *  *    * ******* **  * ****  * ************ ****

gi|26327464|dbj|AK031609.1|     CTGGCTGCGGCTTCCTCGGGGTCTACCACATTGGCGTGGCCTCCTGCCTC 144
gi|34861241|ref|XM_341960.1|    CTGGCTGCGGCTTCCTCGGGGTCTACCACATTGGAGTGGCCTCCTGCCTC 183
gi|16878146|gb|BC017280.1|BC01  CGGGCTGCGGCTTCCTCGGCGTCTACTACGTCGGCGTGGCCTCCTGCCTC 298
                                * ***************** ****** ** * ** ***************

gi|26327464|dbj|AK031609.1|     CGTGAGCACGCGCCCTTCCTGGTGGCCAACGCCACTCACATCTACGGAGC 194
gi|34861241|ref|XM_341960.1|    CGTGAGCACGCGCCCTTCCTGGTGGCCAACGCCACTCACATCTACGGAGC 233
gi|16878146|gb|BC017280.1|BC01  CGCGAGCACGCGCCCTTCCTGGTGGCCAACGCCACGCACATCTACGGCGC 348
                                ** ********************************** ********** **

gi|26327464|dbj|AK031609.1|     CTCGGCAGGGGCGCTCACCGCCACAGCGCTGGTCACTGGGGCCTGCCTGG 244
gi|34861241|ref|XM_341960.1|    CTCGGCAGGGGCGCTTACCGCCACAGCGCTGGTCACTGGGGCCTGCCTGG 283
gi|16878146|gb|BC017280.1|BC01  CTCGGCCGGGGCGCTCACGGCCACGGCGCTGGTCACCGGGGTCTGCCTGG 398
                                ****** ******** ** ***** *********** **** ********

gi|26327464|dbj|AK031609.1|     GTGAAGCAGGTGCCAACATTATTGAGGTGTCCAAGGAGGCCCGGAAGCGG 294
gi|34861241|ref|XM_341960.1|    GCGAAGCGGGTGCCAACATTATTGAGGTGTCCAAGGAGGCTCGGAAGCGG 333
gi|16878146|gb|BC017280.1|BC01  GTGAGGCTGGTGCCAAGTTCATTGAGGTATCTAAAGAGGCCCGGAAGCGG 448
                                * ** ** ********  * ******** ** ** ***** *********

gi|26327464|dbj|AK031609.1|     TTCCTGGGTCCTCTGCATCCCTCCTTCAACCTGGTGAAGACCATCCGTGG 344
gi|34861241|ref|XM_341960.1|    TTCCTGGGTCCCCTGCACCCCTCCTTCAACCTGGTAAAGACCATCCGTGG 383
gi|16878146|gb|BC017280.1|BC01  TTCCTGGGCCCCCTGCACCCCTCCTTCAACCTGGTAAAGATCATCCGCAG 498
                                ******** ** ***** ***************** **** ******  *

gi|26327464|dbj|AK031609.1|     CTGTCTACTAAAGACCCTGCCTGCTGATTGCCATGAGCGCGCCAATGGAC 394
gi|34861241|ref|XM_341960.1|    TTGTCTACTGAAGACCCTGCCTGCTGATTGCCACACGCGTGCCAGCGGAC 433
gi|16878146|gb|BC017280.1|BC01  TTTCCTGCTGAAGGTCCTGCCTGCTGATAGCCATGAGCATGCCAGTGGGC 548
                                 *  ** ** ***  ************* ****   **  ****  ** *

gi|26327464|dbj|AK031609.1|     GCCTGGGCATCTCCCTGACTCGTGTTTCAGACGGAGAGAACGTCATCATA 444
gi|34861241|ref|XM_341960.1|    GCCTGGGCATCTCCCTGACTCGAGTTTCGGATGGAGAGAATGTCATCATA 483
gi|16878146|gb|BC017280.1|BC01  GCCTGGGCATCTCCCTGACCCGCGTGTCAGACGGCGAGAATGTCATTATA 598
                                ******************* ** ** ** ** ** ***** ***** ***
```

Figure 20. (2 of 4)

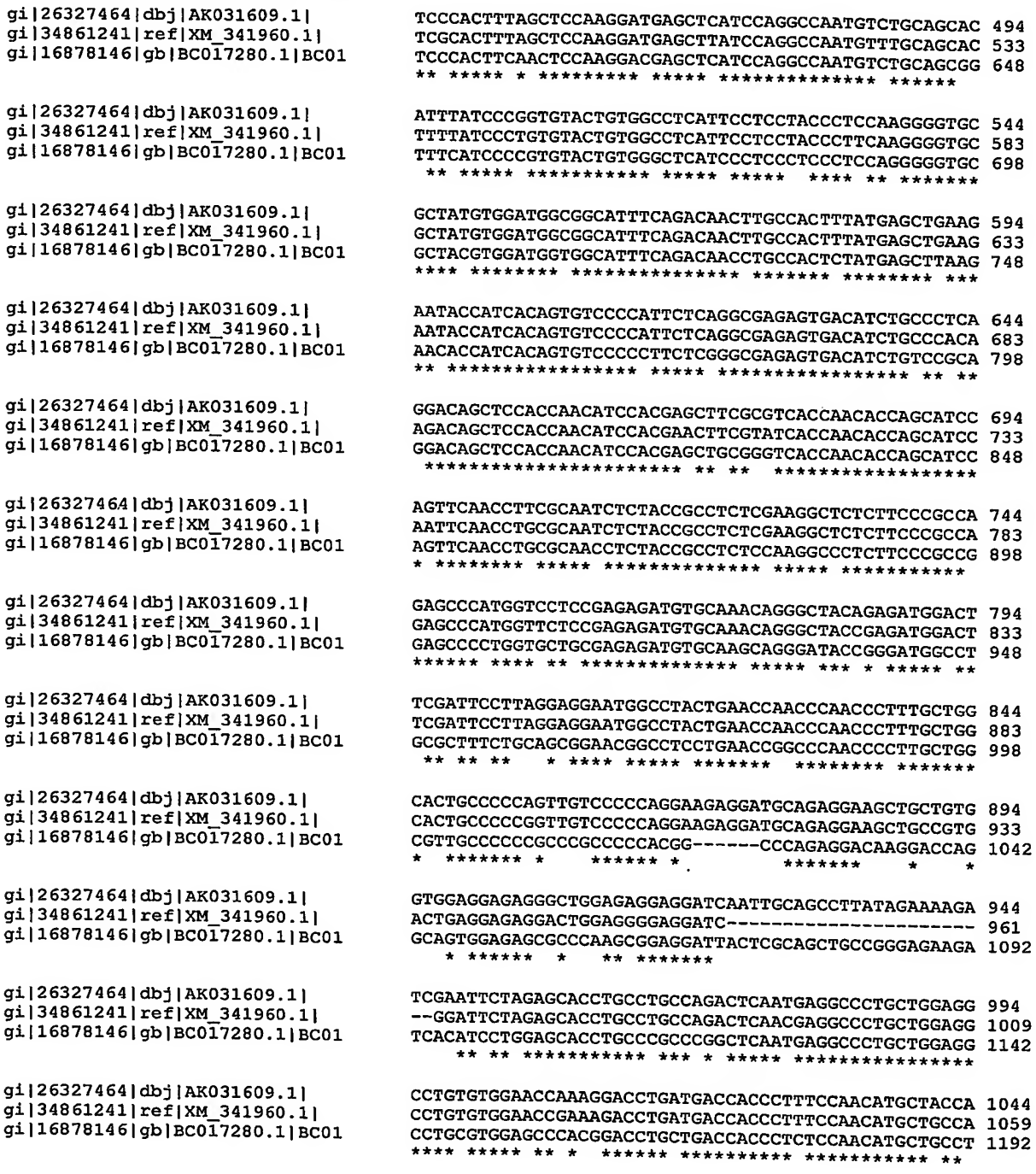


Figure 20. (3 of 4)

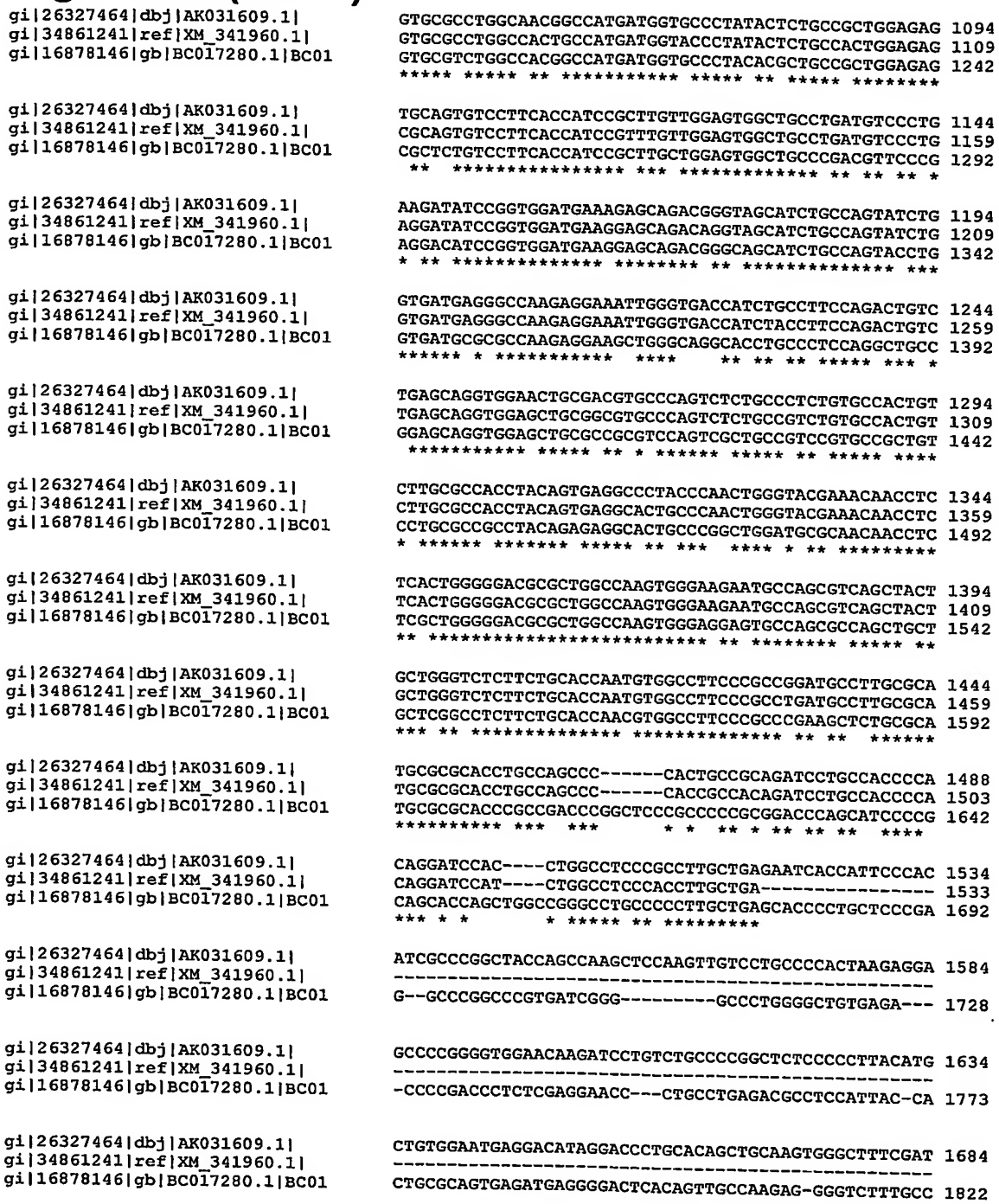


Figure 20. (4 of 4)

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gi|26327464|dbj|AK031609.1|        GTGAAACCTTTCACCAGCCACTCACTATGCTACTCCTGGTGGGGAGGGAT 1734
gi|34861241|ref|XM_341960.1|       ------------------------------------------------- 
gi|16878146|gb|BC017280.1|BC01     GTGGGCCCCCTCGCCAGCCACTCACCA-GCTGCATGCACTGAGAGGGGAG 1871

gi|26327464|dbj|AK031609.1|        GGGGAGTCGCCCTCCCCCGGAGCCCACAGAGCCCTCCCCCGTCACGTC-- 1782
gi|34861241|ref|XM_341960.1|       ------------------------------------------------- 
gi|16878146|gb|BC017280.1|BC01     GTTTCCACACCCCTCCCCTGGGCCGCTGAGGCCCCGCGCACCTGTGCCTT 1921

gi|26327464|dbj|AK031609.1|        ACCTGTGCCTTACTCCTGCCCACCA--CCTTTTCAGTGCAGGGTCAGTCT 1830
gi|34861241|ref|XM_341960.1|       ------------------------------------------------- 
gi|16878146|gb|BC017280.1|BC01     AATCTTCCCTCCCCTGTGCTGCCCGAGCACCTCCCCCGCCCCTTTACTCC 1971

gi|26327464|dbj|AK031609.1|        TAAGAACTCCACATCTGCTGCTGC-TCCCTGGTGTCCAAGTTTCCTTGCA 1879
gi|34861241|ref|XM_341960.1|       ------------------------------------------------- 
gi|16878146|gb|BC017280.1|BC01     TGAGAACTTTGCAGCTGCCCTTCCCTCCCCGTTTTTCATGGCCTGCTGAA 2021

gi|26327464|dbj|AK031609.1|        GA--GTGTGTGAAGAATTATTTATTTTTGCCAAAGCAGATCTAATAAAAG 1927
gi|34861241|ref|XM_341960.1|       ------------------------------------------------- 
gi|16878146|gb|BC017280.1|BC01     ATATGTGTGTGAAGAATTATTTATTTTCGCCAAAGCACATGTAATAAATG 2071

gi|26327464|dbj|AK031609.1|        CCACAGCTCAGCTTCTGCCTTCCTCACTTCTGCATGCT---------- 1965
gi|34861241|ref|XM_341960.1|       ------------------------------------------------- 
gi|16878146|gb|BC017280.1|BC01     CTGCAGCCCAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAA 2121

gi|26327464|dbj|AK031609.1|        -
gi|34861241|ref|XM_341960.1|       -
gi|16878146|gb|BC017280.1|BC01     A 2122
```